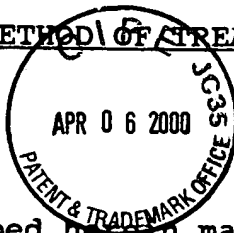


COMPOSITION AND METHOD OF TREATING HEPATITIS C



I. GOVERNMENT INTEREST

This invention described herein may be manufactured, used and licensed by or for the Government for governmental purposes without the payment to us of any royalties thereon.

II. RELATED APPLICATION

This application is a continuation-in-part of U.S. Patent Application Serial No. 08/404,844 filed January 24, 1994, which is a continuation of U.S. Patent Application Serial No. 07/878,372 filed May 4, 1992 [which in turn is a continuation in part of U.S. Patent Application Serial No. 07/759,544, filed September 13, 1991.

III. FIELD OF INVENTION

This invention relates generally to the pharmacological treatment of hepatitis C virus infection in patients.

IV. DESCRIPTION OF THE RELATED ART

Hepatitis C Virus (HCV), the putative agent in the majority of post-transfusion acquired hepatitis, has been recently defined by a new serologic assay. Kuo, G., et al., Science, 244:362-4 (1989). Despite improvement in the quality of the blood-donor pool and the recent implementation of testing of donated blood, the current estimated incidence of acute infection

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1 among persons receiving transfusions is 5 to 10%.

2 Alter, H.J., in Zuckerman, A.J., ed., Viral Hepatitis  
3 and Liver Disease, Allen K. Liss, New York, 1988,  
4 pp.537-42. Chronic hepatitis develops in at least half  
5 the patients with acute HCV infection (representing  
6 about 90% of patients with non-A, non-B hepatitis  
7 (NANB)), and cirrhosis develops in at least 20% of this  
8 group. Thus, of the approximately 3 million persons  
9 who receive transfusions in the United States each  
10 year, acute hepatitis C will develop in about 150,000.  
11 Chronic hepatitis C will develop in at least 75,000 of  
12 these, and among them cirrhosis will develop in more  
13 than 15,000. Among patients with post-transfusion  
14 hepatitis, up to about 90% are positive for the HCV  
15 antibody. Davis, G.L., et al., New England Journal of  
16 Medicine, 321:1501-6 (1989). Patients with sporadic  
17 NANB hepatitis (no specific risk factors) are also very  
18 likely to have the anti-HCV antibody. Kuo, et al.  
19 (1989) above. While most of the patients who contract  
20 hepatitis C will have subclinical or mild disease,  
21 approximately 50% will progress to a chronic disease  
22 state characterized by fluctuating serum transaminase  
23 abnormalities and inflammatory lesions on liver biopsy.  
24 By some estimates, cirrhosis will develop in up to  
25 about 20% of this group. Koretz, R.L., et al.,  
26 Gastroenterology, 88:1251-4 (1985).

1           With the aim of halting or slowing the progression  
2 of HCV-related diseases, a variety of drugs have been  
3 evaluated in recent years. Both acyclovir and  
4 corticosteroids (which are beneficial in autoimmune  
5 chronic active hepatitis) are ineffective. Pappas,  
6 S.C., J. Med. Virol., 15:1-9 (1985); Stokes, P., et  
7 al., Gastroenterology, 92:1783 abstract (1987).

8           To date,  $\alpha$ -interferon (IFA) appears to be the most  
9 promising candidate, although not necessarily the final  
10 answer. Hoofnagle, J.H., et al., in Viral Hepatitis:  
11 1981 International Symposium, Philadelphia, Franklin  
12 Institute Press, 1982, pp. 573-83; Hoofnagle, J.H., et  
13 al., New England Journal of Medicine, 315:1575-8  
14 (1986); Thomson, J., Lancet, 1:539-41 (1987); Kiyosawa,  
15 K., et al., in Zuckerman, A., ed., Viral Hepatitis and  
16 Liver Disease, Allen K. Liss, New York, 1983, pp. 895-  
17 7. Hoofnagle, J.H., et al., Sem. Liver dis., 9:259-263  
18 (1985). The interferons are host proteins made in  
19 response to viral infections as well as other antigenic  
20 stimuli. They are classified by their cell or origin  
21 as well as their antigenicity.  $\alpha$ -Interferon is made by  
22 lymphoblastoid cells,  $\beta$ -interferon by fibroblasts, and  
23  $\gamma$ -interferon by T-cells. Subtypes in each group are  
24 based on antigenic/structural characteristics.  
25 Recombinant forms for each group have been developed  
26 and are commercially available. A pilot study

1 utilizing IFA on ten patients with well-characterized  
2 post-transfusion NANB hepatitis was reported in 1986 by  
3 Hoofnagle et al. (Hoofnagle, J.H., et al., New England  
4 Journal of Medicine, 315:1575-8 (1986)). In this  
5 study, eight of ten patients improved their serum  
6 alanine transaminase (ALT) levels within one month of  
7 starting therapy. IFA therapy consisted of 5 million  
8 units (MU) daily in seven of the patients and one MU  
9 daily in three patients. In all subjects the dose was  
10 gradually reduced to 1 MU daily and then finally  
11 switched to an alternate day or every three day  
12 regimen. In three patients who had post-treatment  
13 liver biopsies, the specimen showed a marked  
14 improvement in the degree of portal inflammation and  
15 loss of parenchymal hepatocytic necrosis. Side effects  
16 were common at the 5 MU/day dose and virtually absent  
17 at 1 MU/day.

18 The effects of recombinant human interferon  $\alpha$  in a  
19 prospective, randomized, double-blind, placebo-  
20 controlled trial in patients with well-documented  
21 chronic HCV infection has recently been carried out.  
22 Di Bisceglie, A.M., et al., New England Journal of  
23 Medicine, 321:1506-10 (1989). Forty-one patients were  
24 enrolled in the trial, 37 of whom were later found to  
25 have antibody to HCV. Twenty-one patients received  
26 interferon  $\alpha$  (2 MU) subcutaneously three times weekly

1 for six months, and twenty received placebo. The mean  
2 serum ALT and the histological reatures of the liver  
3 improved significantly in the patients treated with  
4 interferon, but not in the patients given placebo. Ten  
5 patients treated with interferon (48%) has a complete  
6 response, defined as a decline in mean serum ALT to the  
7 normal range during therapy; three others had a  
8 decrease in mean ALT of more than 50%. After treatment  
9 ended, however, serum ALT usually returned to  
10 pretreatment levels; six to twelve months after the  
11 discontinuation of interferon therapy, only two  
12 patients (10%) still had normal values. The authors  
13 concluded that interferon  $\alpha$  therapy is beneficial in  
14 reducing disease activity in chronic hepatitis C;  
15 however, the beneficial responses are often transient  
16 and side effects are known to appear.

17 In another, broader study, chronic hepatitis C  
18 (NANB hepatitis) is 166 patients was treated with  
19 either 3 MU or 1 MU of recombinant human  $\alpha$ -IFA three  
20 times weekly for 24 weeks or to no treatment. The  
21 serum ALT level became completely normal in 22 of the  
22 26 patients (85%) who responded to treatment with 3 MU  
23 of interferon, and nine of the sixteen patients (56%)  
24 responded to treatment with 1 MU. The patients who  
25 received 3 MU of interferon had histologic improvement  
26 because of the regression of lobular and periportal

[illegible]

10 A multi-center randomized control trial of  
11 recombinant human  $\alpha$ -IFN in patients with chronic NANB  
12 hepatitis has been reported recently. Marcellin, P.,  
13 et al., Hepatology, 13:393-97 (1991). Patients were  
14 randomly assigned to no treatment or to 1 to 3 MU of  $\alpha$ -  
15 interferon given three times a week for 24 weeks.  
16 Forty-five patients (75%) were positive for antibody to  
17 HCV. During the 24 week treatment period, mean serum  
18 ALT levels decreased in both treatment groups, but the  
19 decrease was statistically significant only in the 3 MU  
20 group. However, at 24 weeks, the proportion of  
21 patients with normal ALT levels was similar in the 3 MU  
22 group (39%) and the 1 MU group (45%) and both were  
23 significantly higher than in controls (0%). Repeat  
24 liver biopsy specimens showed a significant decrease in  
25 the severity of histological changes in the higher dose  
26 group but not in the lower dose group or in controls.

1 However, after treatment, the mean ALT levels rose in  
2 both treated groups. The proportion of patients with  
3 normal ALT levels at week 48 was 28% in the 3 MU group  
4 and 20% in the 1 MU group. The authors conclude that a  
5 dose of 3 MU was superior to 1 MU of  $\alpha$ -interferon given  
6 three times per week for 24 weeks in inducing  
7 improvements in serum ALT levels and liver histological  
8 examinations. However, relapse in disease activity  
9 occurred in approximately half of the responders when  
10 interferon was stopped. The response to  $\alpha$ -interferon  
11 did not correlate with the source of infection or  
12 with the presence or absence of anti-HCV antibody titres  
13 in patient sera.

14 It is clear, therefore, that while  $\alpha$ -interferon  
15 has a beneficial effect on the course of HCV infection,  
16 this effect is frequently only transient. therefore,  
17 new modalities are necessary in order permanently to  
18 eradicate the effects of hepatitis C virus on the  
19 patient.

20 Another class of polypeptide immune modifiers  
21 derived from the thymus gland, the thymosins, has been  
22 shown to trigger maturational events in lymphocytes, to  
23 augment T-cell function and to promote reconstitution  
24 of immune defects. Low, T.L.K., et al., "Thymosins:  
25 Structure, Function and Therapeutic Application",  
26 Thymus, 6:27-42 (1984).

1           Thymosin Fraction Five (TF-5), originally  
2   described by Goldstein et al. (Proc. Nat'l Acad. Sci.  
3   (USA), 69:1800-1803 (1972)), is a partially purified  
4   extract of bovine thymus containing at least 40 peptide  
5   components, 20 of which have been purified to  
6   homogeneity or near homogeneity; it contains about 0.6%  
7   of Thymosin  $\alpha$ -1 (THN $\alpha_1$ ). Low, 1984, above.

8           THN $\alpha_1$ , initially isolated from TF-5, has been  
9   sequenced and chemically synthesized. Wetzell, R., et  
10   al., Biochemistry, 19:6096-6104 (1980). Its sequence  
11   is highly homologous in mice, calves and humans. THN $\alpha_1$   
12   is a 28 amino acidic polypeptide with a molecular  
13   weight of 3100 that has shown activity qualitatively  
14   similar to TF-5 in modulating the immune system. Low,  
15   T.L.K., et al., J. Biol. Chem., 254:981-6 (1979).  
16   THN $\alpha_1$  has potent immunologic activity, including  
17   stimulation of  $\alpha$ - and  $\gamma$ -interferon production,  
18   increasing macrophage migration inhibitory factor  
19   production, inducing expression of T-cell markers,  
20   including IL-2 receptors, and improving T-cell helper  
21   cell activity. Schulz, R.S., et al., in The  
22   Lymphocyte, Allen J. Liss Inc., New York, 1981, pp.  
23   191-215; Low, T.L.K., et al., in "Thymosins: Structure,  
24   Function and Therapeutic Applications", Thymus, 6:27-43  
25   (1984); Koutab, N.M., et al., Immunopharm., 16:97-105  
26   (1988). Studies in mice have demonstrated a



1 synergistic effect of  $\text{THN}\alpha_1$  and interferon on natural  
2 killer-cell activity in immunosuppressed mice.  
3 Favilli, C., et al., Cancer Immunol. Immunother.,  
4 20:189-92 (1985). TF-5 and  $\text{THN}\alpha_1$  can influence  
5 immunoregulatory T-cell function, promote production of  
6 interferon- $\alpha$ , interferon- $\gamma$  and interleukin-2 by human  
7 lymphocytes and increase interleukin-2 receptor  
8 expression. Marshall, G. D., et al., J. Immunol.,  
9 126:741-4 (1981); Mutchnick, M.G., et al., Clin.  
10 Immunol. Immunopathol., 23:626-33 (1982); Sztejn, M.B.,  
11 et al., Proc. Nat's Acad. Sci. (USA), 83:6107-6111  
12 (9186); Serrate, S.A., et al., J. Immunol., 1939:2338-  
13 43 (1987); Bazevanis, C.N., et al., Immunopharm.,  
14 13:133-41 (9187); and, Svedersky, L.P., Eur. J.  
15 Immunol., 12:244-7 (1982).

16 Clinical trails of TF-5 and  $\text{THN}\alpha_1$  as primary or  
17 adjunctive therapy in patients iwth immunodeficiency or  
18 cancer indicate that these agents enhance immune  
19 responsiveness and augment specific lymphocyte  
20 functions. Clinical trials of TF-5 and purified  $\text{THN}\alpha_1$   
21 have been underway for a number of years. Early trials  
22 in patients with cancer or immunodeficiency states were  
23 encouraging, though not definitive. Goldstein, A.L.,  
24 et al., Transp. Proc., 9:1141 (1977); Barrett, D.J., et  
25 al., J. Pediatr., 97:61 (1980); and Cohen, M.H., et  
26 al., J. Amer. Med. Assoc., 241:1813-5 (1979).  $\text{THN}\alpha_1$

1 use has been described in a randomized trial of  
2 patients with nonsmall cell lung cancer. Patients were  
3 treated with  $\text{THN}\alpha_1$  at a dose of 900  $\mu\text{grams}/\text{m}^2$   
4 subcutaneously twice weekly or daily for two weeks and  
5 then twice weekly after completing a course of  
6 radiotherapy. The only side effect of  $\text{THN}\alpha_1$  was mild  
7 burning at the injection site in three patients. This  
8 was attributed to the drug lot and may have been due to  
9 the carrier preparation. Relapse-free survival and  
10 overall survival were greater in both  $\text{THN}\alpha_1$  treatment  
11 groups than in the placebo group; some restoration of  
12 radiation-suppressed immune function was also noticed.  
13 There was no increase in T-cell numbers associated with  
14 this. Schulof, R.S., et al., J. Biol. Response  
15 Modifiers, 4:147-58 (1985).

16 Recent double-blind, randomized trials with  
17 thymosins have been performed in elderly men in an  
18 effort to increase response to influenza vaccine.  
19 Gravenstein, S., et al., JAGS, 37:1-8 (1989). Patients  
20 received synthetic  $\text{THN}\alpha_1$  subcutaneously twice weekly  
21 starting at the time the influenza vaccine was given.  
22 At six weeks post-vaccine, those patients randomized to  
23 receive the drug had higher levels of antibody to  
24 influenza than controls. This difference was  
25 accentuated in the very elderly (ages 77-99). No

1 clinical or biochemical toxicity was observed in drug  
2 recipients.

3       There are preliminary reports that thymosins may  
4 be effective against infections caused by hepatitis  
5 viruses other than HCV. In an animal model of viral  
6 hepatitis, the woodchuck infected with the Woodchuck  
7 Hepatitis Virus, THN $\alpha_1$ , suppressed viral DNA  
8 replication, but produced no improvement in clinical  
9 parameters. Korba, B.E., et al., Hepatology, 12:Abs.  
10 880 (1990). In a pilot clinical trial with patients  
11 with Chronic Active Hepatitis B caused by the hepatitis  
12 B virus (HBV), patients treated for a year with THN $\alpha_1$ ,  
13 (5 patients) or with TF-5 (2 patients) showed a marked  
14 decrease in serum ALT; 6 of the 7 patients also showed  
15 reduced levels of serum HBV DNA, and 5 of 6 patients  
16 initially positive for serum hepatitis B surface  
17 antigen (HBsAg) subsequently cleared this antigen.  
18 Mutchnick, M.C., et al., Hepatology, 10:Abs. 575  
19 (1989). No suggestion was made in these abstracts that  
20 the thymosins would be effective against any other  
21 hepatitis viruses.

22       There remains, therefore, an important need in the  
23 art for a new modality for the treatment of HCV  
24 infections in mammals; this modality is disclosed  
25 below.

26                   SUMMARY OF THE INVENTION

1           A treatment modality for HCV infections has been  
2   devised comprising the administration to mammals of  
3   immune system-potentiating doses of one or more  
4   thymosins in combination with interferon therapy.

5           It is thus an object of this specification to  
6   disclose compositions and methods for the treatment of  
7   acute or chronic HCV infections in mammals comprising  
8   (combination therapy with one or more thymosins and one  
9   or more interferons.

10          This and other objects will become apparent by  
11   reference to the specification and to the appended  
12   claims.

13                           DESCRIPTION OF THE INVENTION

14          A novel modality for treating HCV infection in  
15   mammals has been devised, comprising the administration  
16   to such mammals of one or more thymosins at doses which  
17   potentiate immune responses, in combination with anti-  
18   viral doses of one or more interferons.

19          By the term "thymosins" is meant any or all of the  
20   immune system potentiating polypeptides naturally  
21   occurring in the thymus gland or produced by chemical  
22   or recombinant means, or fragments derived from any of  
23   these polypeptides. By the term "mammals" is meant any  
24   mammalian subject, including human and animal patients,  
25   requiring treatment for hepatitis C infection.

26   "Mammal" and "subject" are used interchangeably.

1 Thymosin preparations suitable for treating HCV  
2 infections include TF-5,  $\text{THN}\alpha_1$  and fragments thereof,  
3 e.g., C-terminal 4-28 and 15-28, and N-terminal 1-8, 1-  
4 14 and 1-20 fragments. These may be obtained from  
5 Alpha-1 Biomedicals Inc., Foster City, California.

6 Subjects, e.g., human patients, may receive the  
7 thymosin by subcutaneous injection or infusion, at  
8 appropriate intervals for an appropriate period of  
9 time. The thymosin is administered to mammals infected  
10 with hepatitis C virus in amounts which facilitate or  
11 promote in vivo inactivation of hepatitis C virus. A  
12 pharmaceutical dosage unit of an immune system-  
13 potentiating amount of a thymosin, such as TF-5, can be  
14 from about 900 to about 1200 mg/m<sup>2</sup> body surface area in  
15 a pharmaceutically acceptable carrier. A  
16 pharmaceutical dosage unit of an immune system-  
17 potentiating amount of a thymosin, such as  $\text{THN}\alpha_1$  or  
18 immune system-potentiating fragments thereof, can be  
19 from about 900 to about 1200  $\mu\text{g}/\text{m}^2$  body surface area in  
20 a pharmaceutically-acceptable carrier. Lyophilized  
21 preparations of thymosins or fragments which contain  
22 mannitol and phosphate buffer are dissolved in diluent  
23 period to dispensing. Thymosins in diluent should  
24 remain stable for at least six months when stored in a  
25 refrigerator. It is convenient to dispense thymosin  
26 solutions in one ml dose vials per month.

1 For a thpical human patient, an administration  
2 regimen of twice weekly (e.g., Monday and Thursday)  
3 subcutaneous injection of about 1500 to about 1700  $\mu$ g  
4 of THN $\alpha_1$  or fragments therefrom is convenient. Dosages  
5 and length of treatment can be flexible, and can be  
6 determined by the subject's clinical response to the  
7 thymosins.

8 The course of the disease and its response to drug  
9 treatments may be followed by clinical examination and  
10 laboratory findings. As elevated serum alanine  
11 aminotransferase (ALT) and aspartate aminotransferase  
12 (AST) are known to occur in uncontrolled hepatitis C,  
13 and as a complete response to treatment is generally  
14 defined as the normalization of these serum enzymes,  
15 particularly ALT (Davis, G.L., et al., New England  
16 Journal of Medicine, 321:1501-6 (1989)), progress of  
17 treatment with thymosins is conveniently followed by  
18 this art-recognized test performed, e.g., on a  
19 sequential multiple analyzer.

20 Another means of evaluating subjects having  
21 antibodies to HCV (not all subjects with hepatitis C  
22 have detectable antibody to HCV - Weiner, A.J., et al.,  
23 Lancet, 335:1-3 (1990)) is to periodically test  
24 subjects' sera for the titer of these antibodies.  
25 Anti-HCV antibodies may be tested by the currently  
26 available C 100-3 test (Kuo, G., et al., Science,

1 244:362-4 (1989)), by an Elisa test (Ortho Diagnostic  
2 Systems, Raritan, N.J.) or by a recombinant assay  
3 (RIBA-1 and RIBA-2, Chiron Corporation, Emeryville,  
4 CA). Any suitable test may be used.

5 In order to follow the course of HCV replication  
6 in subjects in response to drug treatment, HCV RNA may  
7 be measured in serum samples by, for example, a nested  
8 polymerase chain reaction assay that uses two sets of  
9 primers derived from the NS3 and NS4 non-structural  
10 gene regions of the HCV genome. Farci, P., et al., New  
11 England Journal of Medicine, 325:98-104 (1991); Ulrich,  
12 P.P., et al., J. Clin. Invest., 86:1609-14 (1990).

13 Other appropriate laboratory tests to follow the  
14 course of treatment are listed in Example 1 below.

15 Thymosin therapy is preferably used in combination  
16 with interferon therapy, thereby combining the immune  
17 system potentiating effect of thymosins with the anti-  
18 viral effects of the interferons. An improved response  
19 rate at the currently used interferon doses would be  
20 beneficial, particularly in the light of dose-limiting  
21 side effects at higher doses of these proteins. An  
22 offshoot of this concept is the ability to achieve  
23 comparable efficacy with interferon plus thymosin at  
24 lower doses than would be required with interferon  
25 alone.

1           In this combination therapy regimen, one or more  
2   interferons (for example, recombinant interferon  $\alpha$ -2b,  
3   Intron-A, Schering-Plough, Kenilworth, New Jersey) is  
4   (are) administered subcutaneously to subjects, e.g.,  
5   human patients, at doses ranging between about 1 MU and  
6   3 MU along with or sequentially with one or more  
7   thymosins, preferably including THN $\alpha_1$ , at a dose of  
8   about 900 to about 1200  $\mu\text{g}/\text{m}^2$  body surface area.

9           Although the example above speaks in terms of  
10   recombinant interferon  $\alpha$ -2b, other anti-HCV-effective  
11   interferons such as  $\alpha$ -,  $\beta$ - and  $\gamma$ -interferons,  
12   recombinant or naturally occurring, may be  
13   advantageously used in this invention.

14           This combination dose regimen is flexible, and  
15   depends on the clinical condition of the subject.  
16   Where subjects are refractory to the preferred dosage  
17   levels, these may be increased within the limits  
18   dictated by undesirable side effects. Typically,  
19   injections are made five times per week and continue  
20   until an acceptable response by the subject is  
21   realized.

22           Tests to determine the effectiveness of the  
23   combination therapy may be the same as those described  
24   above for thymosin treatment alone. In addition,  
25   histological examination of liver biopsy samples may be  
26   used as a second major criteria for evaluation.



1 Knodell, R.G., et al., Hepatology, 1:431-5 (1981),  
2 whose Histological Activity Index (portal inflammation,  
3 piecemeal or bridging necrosis, lobular injury and  
4 fibrosis) provides a scoring method for disease  
5 activity.

6 The following examples are provided merely to  
7 illustrate the invention, and are not to be construed  
8 in any way as limiting the scope of invention as set  
9 forth in the specification and claims.

10 EXAMPLE 1

11 Preparation of Injectable Formulation

12 Pharmaceutical dosage units or 1 ml each are  
13 prepared from the ingredients shown in Table 1 below.

14 TABLE 1

| 15 | <u>Active Ingredient</u>    | <u>Amount Per mL</u> |
|----|-----------------------------|----------------------|
| 16 | Thymosin $\alpha$ -1        | 0.0016 g             |
| 17 | <u>Inactive Ingredients</u> |                      |
| 18 | mannitol, U.S.P.            | 0.050 g              |
| 19 | sodium phosphate dibasic,   |                      |
| 20 | heptahydrate, U.S.P.        | 0.002 g              |
| 21 | sodium phosphate monobasic, |                      |
| 22 | monohydrate, U.S.P.         | 0.0005 g             |
| 23 | sodium phosphate dibasic,   |                      |
| 24 | 2 mg/ml solution            |                      |
| 25 | sodium phosphate monobasic, |                      |
| 26 | 0.5 mg/ml solution          |                      |
| 27 | water for injection, U.S.P. |                      |
| 28 |                             |                      |

EXAMPLE 2

Treatment of Hepatitis C Infections in  
Human Patients with Thymosins and Interferons

Adult patients with chronic active hepatitis C (CAHC) are randomized to one of four study groups, made up of about 40 patients per group. Selection criteria include: (1) patients are adults (at least 18 years of age); (2) serum ALT is elevated for at least six months prior to treatment iwth at least one value greater than twice the upper limit of normal in the laboratory doing the testing; (3) patients test positive for HCV antibody on two occasions andon a confirmatory test; and (4) liver biopsy within three months of treatment exhibits pathology consistent with chronic active hepatitis.

Exclusion criteria include: (1) recent use of other anti-viral or immunosuppressive medication; (2) hemophilia, pregnancy or HIV invecton, or other serious illness that could prevent completion of the course of treatment; (3) other forms of liver disease, including hepatitis A or B,  $\alpha$ -1 antitrypsin deficiency, Wilson's disease, and hemochromatosis must be absent; (4) autoimmune markers (ANA, ASMA, AMA, anti-LKMI) must be absent or, if present, titers should be  $< 1:40$ ; (5) leukocyte deficiency ( $< 3,000$ ); (6) low absolute neutrophil count ( $< 1,000$ ); (7) low platelets ( $< 75,000$ );

1 (8) low Hb (<11 g/dL); (9) high bilirubin (>4 mg/dL);  
2 and (10) low serum albumin (3 g/dL).

3 The first of the four randomized groups receives  
4 interferon, preferably interferon  $\alpha$ -2b, at a dose of 3  
5 million units (MU) subcutaneously (SQ) on Mondays,  
6 Wednesdays and Fridays, and receives placebos on  
7 Tuesdays and Saturdays. The second group receives the  
8 same dose/schedule of interferon, plus a thymosin,  
9 preferably THN $\alpha_1$ , at a dose of 900  $\mu$ g/m<sup>2</sup> SQ on Tuesdays  
10 and Saturdays. The third group receives the same  
11 dose/schedule of a thymosin alone. The fourth group  
12 receives placebo treatment initially, but can be  
13 randomized to the three treatment groups thereafter.  
14 Interferons and thymosins can be recombinant.

15 Patients begin treatment while hospitalized for  
16 about one week, during which period side-effects are  
17 monitored.

18 Outpatient follow-up is initially at one week  
19 intervals for two weeks, then at two week intervals for  
20 two months, and then monthly for the remainder of the  
21 treatment period. At each visit the following lab  
22 tests are performed: CBC, platelet count, differential  
23 and ESR, ALT, AST, GGT, alkaline phosphatase,  
24 bilirubin, total bilirubin/albumin and HCV antibody.  
25 At monthly intervals serum  $\gamma$ -globulin, TSH, ANA and  
26 ASMA are assessed.

1 Drug toxicity is monitored on an ongoing basis  
2 using both clinical and laboratory parameters.

3 Within one month of completing the initial six  
4 months of treatment, patients undergo liver biopsy for  
5 pathological examination according to Knodell et al.  
6 above. This system provides a numerical scoring system  
7 of histological activity in patients with asymptomatic  
8 CAH.

9 At this time, control patients are randomized into  
10 three groups to receive one of the three treatment  
11 modalities, assuming that they still have CAH on  
12 follow-up liver biopsy, and that one arm of the study  
13 does not show highly significant positive or negative  
14 results on analysis at six months.

15 Patients in the treatment groups are followed to  
16 evaluate recrudescence of disease as evidenced by  
17 rising ALT levels. Patients who showed response in the  
18 initial six month treatment period, but who have a  
19 recurrence of the disease thereafter, are provided with  
20 additional therapy.

21 Additional serum or tissue tests are performed if  
22 possible: evaluation of antibodies to interferons and  
23 thymosins, polymerase chain reaction amplification of  
24 hepatitis C genome segments in liver biopsy samples,  
25 and quantitative evaluation of anti-hepatitis C serum  
26 titers.

EXAMPLE 3

The treatment protocol is as in Example 2, except that the interferon is used at the level of 2 MU, and the thymosin at 1050  $\mu\text{g}/\text{m}^2$ .

EXAMPLE 4

The treatment protocol is as in Example 3, except that 1 MU of the interferon and 1200  $\mu\text{g}/\text{m}^2$  of the thymosin are used.

EXAMPLE 5

Analysis of Data

There are two primary criteria for response to therapy- normalization of ALT levels by the end of the treatment period (a partial response may be defined as a decrease of at least 50% of initial ALT), and histological improvement as determined by the Histological Activity Index (HAI) of Knodell et al. above.

This analysis provides a raw score ranging from 1 to 22 per sample. Paired data can be analyzed using the Wilcoxon paired-sample test. Additionally, samples can be classified into mild, moderate or reverse CAH, and improvement assessed using the Chi-square statistical analysis.

Life-table analysis is used to evaluate remission and relapse status in terms of normalization of ALT levels. Other continuous variables are analyzed using